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Colonization of *Ulothrix flacca*, *Urospora penicilliformis* and *Blidingia minima* (Chlorophyta) in Comodoro Rivadavia harbor (Chubut, Argentina)

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Abstract: Coverage of the green algae *Ulothrix flacca*, *Urospora penicilliformis* and *Blidingia minima* was studied on rocky substrata immersed in Comodoro Rivadavia harbor. Colonization was clearly influenced by the level at which stones were immersed. These green algae grew almost exclusively in the intertidal zone, with only traces of *B. minima* and *U. flacca* found on subtidal samples. *B. minima* and *U. flacca* showed no level preferences within the intertidal. *U. penicilliformis* had significantly higher coverage at upper than at middle intertidal levels, but this trend varied seasonally. Colonization was also strongly influenced by the season during which substrata were immersed: *U. penicilliformis* and *U. flacca* reached maximum coverage during spring and *B. minima* during winter. Interactions between intertidal level and season were also highly significant for all three species, meaning that the seasonal cycle of colonization showed different trends at different intertidal levels.

Key words: fouling, *Ulothrix flacca*, *Urospora penicilliformis*, *Blidingia minima*, Patagonia, Argentina

Green algae are important components of fouling communities of harhors throughout the world, colonizing mainly the upper, well-illuminated levels of piers and other structures, as well as the floating line of ships (Bastida, 1971).

Several species belonging to the genera *Ulva*, *Enteromorpha*, *Cladophora*, *Bryopsis* and *Chaetomorpha* have been recorded in different harbors of Buenos Aires Province, such as Mar del Plata (Bastida, 1971; Piriz, 1972; Bastida *et al.*, 1980), Puerto Quequén (Bastida & Brankevich, 1980, 1981, 1982; Brankevich *et al.*, 1984, 1985, 1986, 1988) and Puerto Belgrano (Bastida & Torti, 1973; Bastida *et al.*, 1974), as well as in the intertidal zone of Nuevo Gulf, northern Patagonia (Díaz *et al.*, 2002).

Rico & Lanas (2001) recorded for the first time the occurrence of *Ulothrix flacca* (Dillwyn) Thuret, *Urospora penicilliformis* (Roth) J.E. Areschoug and *Blidingia minima* (Nägeli ex Kützing) Kylin on intertidal hard substrata of Comodoro Rivadavia harbor. These algae had not been previously reported as foulers of Argentine harbors, except for an undetermined species of *Ulothrix*, which was found among the microfouling assemblages of a thermoelectric power station in Puerto Quequén (Bastida & Brankevich, 1980, 1981; Brankevich *et al.* 1986).

The aim of this study is to analyze the colonization pattern of these three species on rocky substrata immersed in Comodoro Rivadavia harbor.

MATERIALS AND METHODS

Study area. Comodoro Rivadavia harbor is located at 45° 52' S, 67° 28' W. It is composed of two kind of structures: an older section of vertical walls, and a new breakwater, which was built using natural rocks and man-made concrete blocks. Tidal amplitude during spring and neap tides are 6.21 m and 4.34 m, respectively (Servicio de Hidrografía Naval, 1998). Since chemical data of seawater within the harbor were not available, salinity was measured on individual samples collected at each season. Salinity varied between 33.4 and 33.8 psu. Mean monthly temperatures of coastal waters near Comodoro Rivadavia varied between 9° C (August-September) and 16° C (February-March) (W. Maza, Center of Climatic Prediction, pers. comm.). A photograph of the study area appeared in a previous paper (Rico *et al.*, 2001).

Sampling and data analysis. As the nature of the substratum can affect both initial colonization and further development of fouling assemblages (Anderson & Underwood, 1994) and intertidal boulders (McGuinnes & Underwood, 1986),

all sampling units consisted of the same granitic stones found in the breakwater. They were originally extracted from Cerro Dragón, Chubut Province (45° 35' S, 69° 04' W). On the other hand, natural rocky reefs around Comodoro Rivadavia are made of sandstone. All the experimental substrata used in this study were devoid of marine life, since they had not been previously submerged in seawater. At the beginning of each season, stones with a roughly square shape (ca. 20 x 20 cm) were collected from the breakwater and glued to the substratum with epoxy putty. They were distributed along 4 transects perpendicular to the breakwater, separated by around 20 m, at 3 levels: upper intertidal, middle intertidal, and subtidal. The upper intertidal was sampled at approximately 4.50 m above mean low water level (MLWL). It was the most elevated zone where macroscopic marine organisms could be found, and was dominated by a belt of green algae. Middle intertidal samples were glued at around 2.60 m above MLWL, in an area characterized by the appearance of several invertebrate taxa. Stones could not be glued at the lowest intertidal level. Subtidal samples were immersed at around 4 m below MLWL.

Subtidal replicates contained only traces of *Blidingia* and *Ulothrix* and were not analyzed in this study. *Urospora* was completely absent at this level. Stones remained immersed 84-100 days, during the following intervals: 19/12/97-29/03/98 (summer), 29/03/98-28/06/98 (autumn), 28/06/98-20/09/98 (winter), 20/09/98-20/12/98 (spring).

A total of 32 intertidal samples (4 seasons x 2 levels x 4 replicates) were analyzed. Two additional stones were glued at each level, anticipating possible losses, but no intertidal replicates were lost during the study period.

Samples were fixed in 4 % formalyn in seawater. Algae were determined under a Zeiss optical microscope.

Coverage of the whole algal assemblage was assessed within a 100 cm^2 surface in the center of each stone by means of a grid of points. Tbe contribution of *Ulothrix*, *Urospora* and *Blidingia* was then estimated counting their thalli under microscope in ten subsamples of known area in order to estimate the percentage cover of each species.

Data were analyzed by two-way ANOVAs (Sokal & Rohlf, 1981). Homogeneity of variances was verified by the Cochran's C test (Winer, 1971). Coverage data of *Blidingia* were fourth root transformed ($x' = x^{0.25}$) in order to achieve homogeneity, but variances were still moderately heterogeneous after transformation (Cochran's C, $P < 0.05$). We believe, however, that in this case significant results for the interaction and the seasonal factor are sufficiently robust, since probability values were far beyond the 0.05 significance level (see Table 1).

Table 1. Two-way ANOVA of percentage cover of *Ulothrix flacca, Urospora penicilliformis* and *Blidingia minima* in Comodoro Rivadavia harbor. Significant results are in bold.

Effect	Degrees of Freedom	Mean Square	F	P
Ulothrix flacca				
Season	3	927.3	4.20	**0.016**
Level	1	897.8	4.06	0.055
Interaction	3	1373.8	6.22	**0.003**
Error	24	221.0		
Cochran's C = 0.351, P > 0.05				
Urospora penicilliformis				
Season	3	747.5	15.04	**0.000010**
Level	1	1906.5	38.35	**0.000002**
Interaction	3	952.8	19.17	**0.000001**
Error	24	49.71		
Cochran's C = 0.341, P > 0.05				
Blidingia minima				
Season	3	1.474	6.03	**0.0033**
Level	1	0.306	1.25	0.2745
Interaction	3	1.937	7.93	**0.0008**
Error	24	0.244		
Fourth root transformation, Cochran's C = 0.495; P < 0.05				

RESULTS

Taking all seasons and intertidal levels together, coverage was 4-5 times higher in *Ulothrix flacca* and *Urospora penicilliformis* than in *Blidingia minima*, mainly because *U. flacca* and *U. penicilliformis* dominated the upper intertidal during spring (Fig. 1).

Coverage of *Ulothrix flacca* (Table 1, Fig. 1) showed non-significant differences between levels, but changed significantly among seasons, being completely absent in summer. The interaction between spatial and temporal factors was highly significant, since coverage was maximum during spring at the upper intertidal, but during winter at the middle intertidal.

All factors of the ANOVA were highly significant for *Urospora penicilliformis* (Table 1, Fig. 1). Coverage was higbest in spring and lowest in autumn, and was significantly higher in the upper than in the middle intertidal. A higbly significant interaction was also detected between both factors, since patterns of ahundance sbowed different trends at both levels: coverage was maximum during spring at the upper intertidal, but during winter at the middle intertidal.

Coverage of *Blidingia minima* (Table 1, Fig. 1) showed non-significant differences between levels. Seasonal changes in coverage were significant, with maximum values during winter and minimum values during summer. Interaction between both factors was also significant, since patterns of abundance showed different trends at both levels: coverage was maximum during autumn at the upper intertidal, but during winter at the middle intertidal.

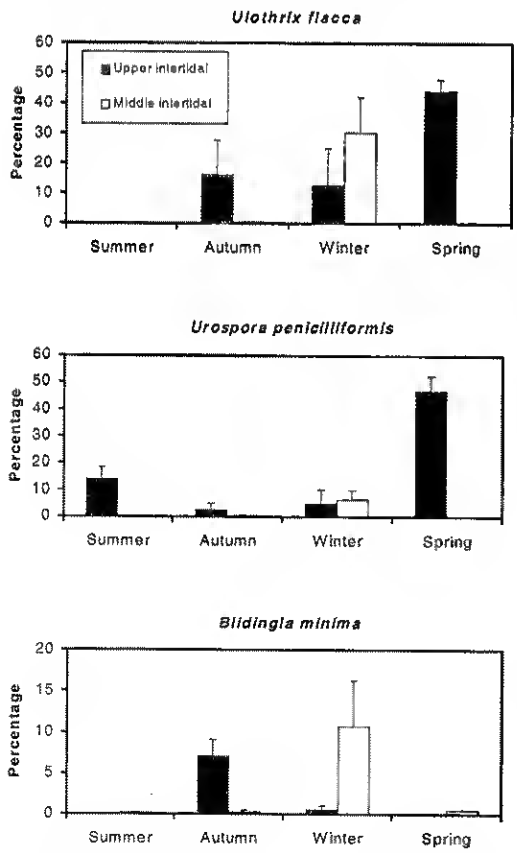

Fig. 1. Percentage cover (mean + standard error) of *Ulothrix flacca*, *Urospora penicilliformis* and *Blidingia minima* at upper and middle intertidal levels of Comodoro Rivadavia harbor at the end of each season. Note the change of scale in *Blidingia minima*.

DISCUSSION

Results of this study show that the colonization pattern of *Ulothrix flacca*, *Urospora penicilliformis* and *Blidingia minima* in Comodoro Rivadavia harbor was clearly influenced by the level at which substrata were immersed, since these species were almost exclusively intertidal. *B. minima* and *U. flacca* showed no significant level preferences within the intertidal zone. On the other hand, *U. penicilliformis* had significantly higher coverage at upper than at middle intertidal levels, but this trend varied seasonally. The upper intertidal has been regarded as the natural habitat of *B. minima* (Bliding, 1963; Boraso, 1970, 1975; Iima, 1989). This study shows, however, that during early stages of development of the fouling community, this species is also abundant at the middle intertidal in protected environments within the harbor, probably as a consequence of reduced wave action.

Colonization of these three algae was also strongly influenced by the season at which substrata were immersed. *U. penicilliformis* and *U. flacca* reached their maximum coverage during spring and *B. minima* during winter. Environmental factors such as air and water temperature, light intensity and photoperiod, are known to have a remarkable influence during the whole life cycle of marine algae, but particularly on early developmental stages (Ohno, 1969). Bischoff & Wiencke (1995) have found that cold-temperate strains of *U. penicilliformis* have an optimum growth temperature between 5 and 15 °C and an upper survival temperature of 24-26 °C. Maximum coverage of *B. minima* occurred in winter. The species was present from autumn to spring, when water temperatures ranged between 9 and 15 °C. This agrees with phenological observations made by

Iima (1989) in Japan, where this species grows abundantly at temperatures of 5-15 °C (spring), dies out at 20 °C (summer) and reappears at temperatures lower than 15 °C (autumn). Both *B. minima* and *U. flacca* disappeared during summer. The abrupt decline in the abundance of intertidal microalgae at all levels during summer has been attributed to intolerance of physiological stresses caused by insolation and desiccation (Underwood, 1984). *B. minima* extended its distribution to higher level of the shore during colder months than during the summer, a fact also reported by Underwood (1981) in algae from an Australian rocky intertidal community. Chapman (2002) has shown that there was considerable variation in patterns of abundance and diversity of assemblages colonizing subtidal boulders submerged at different times. Algal assemblages also varied greatly over short time periods on wave-exposed rocky shores in New South Wales (Underwood & Chapman, 1998a).

Interactions between intertidal level and season were highly significant for all three species, meaning that the seasonal cycle of colonization showed different trends at different intertidal levels. Complex spatial variability and significant interactions between spatial and temporal measures were also common in intertidal assemblages on sheltered rocky shores in Australia (Underwood & Chapman, 1998b). Considerable inconsistency and variation was found as well in rocky intertidal algal assemblages developing in experimentally cleared patches (Chapman & Underwood, 1998).

Further observational and experimental studies will be necessary to understand the relative importance of physical factors, competition and herbivory in controlling the abundance of *Ulothrix flacca*, *Urospora penicilliformis* and *Blidingia minima* in mature stages of succession on both natural and man-made intertidal habitats.

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